Page 1, paragraph beginning on line 26

In general, these peptide analogs, called pseudopeptides, have, as a first advantage, a metabolic stability which is greater than that of natural peptides or proteins because they are not degraded by natural proteases or are degraded less rapidly. Moreover, the conformational changes induced by these chemical modifications can improve the biological properties of these pseudopeptides, see for example the decapeptide analogs which are antagonists of the hypothalmic hormones and which are described in ~~WO-A-92/13883:~~ WO-A-92/13883.

Page 2, insert the following paragraph before the paragraph beginning on line 28

While the techniques for the synthesis of so-called natural peptides, in particular on solid supports, are well established and make it possible to easily prepare peptides comprising several tens of amino acids, the introduction of these modifications in order to prepare pseudopeptides renders the synthesis more complex, in particular for long pseudopeptides.

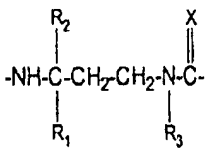
Page 2, paragraph beginning on line 28

Moreover, in the field of immunology and both in the diagnosis of viral or autoimmune diseases and in immunotherapy or vaccination, the synthetic peptides mimicking the epitopes of proteins represent a valuable alternative. The size of the peptides which are analogs of these antigenic determinants or epitopes is an important factor in the choice of these peptides and has been the subject of numerous publications (M.H.V. Regenmortel, Immunology Today, 10(8), p. 266-271, 1989 or M.H.V. Regenmortel, Biomedical Peptides, Proteins & Nucleic Acids, 1, p. 109-116, 1995). While originally it was accepted that an epitope comprises between 15 and 22 amino acids, recent studies show that this size may be reduced to a few amino acids. In the immunity domain, crystallographic studies on the interaction of peptides and the major histocompatibility complex (MHC) indicate a size of 9 to 13 amino acids for a good interaction with the MHC class I molecules and 9 to 25 for the MHC class II (H.G. Rammensee, Current Opinion in Biotechnology, 7, p. 85-96, 1995). Likewise, in diagnosis, the size is a critical factor for the use of peptides. In the case of HIV (human immunodeficiency virus), the smallest epitopes comprise from 4 to 6 amino acids but the peptides used still have a size greater than at least 12 amino acids (D. Osmanov, AIDS, 5(1),

WHO1-WHO9, 1991). In another example, such as the diagnosis of Chagas' disease, the peptides used comprise a minimum of 12 amino acids (WO-A-97/18475). In (Bradshaw C.G. et ~~al.,~~al. (J. Med. Chem., 37, 1991-1995, 1994), fluorescent probes which are analogs of the heptapeptide antagonist of NK_2 were obtained by substitution of an amino acid and coupling with a fluorophore.

Page 3, paragraph beginning on line 1

It is the object of the present invention to describe a novel family of pseudopeptides comprising a novel carbaza unit significantly modifying the peptide backbone and whose use in the context of peptide synthesis is easy both in solid phase and in liquid phase, and this even for peptides of a large size and in particular greater than 6 amino acids. This novel family of pseudopeptides can be used in the diagnostic field to provide in vitro methods for the diagnosis of pathology conditions associated with the presence of endogenous or exogenous proteins in an individual, or in the therapeutic field, and in particular immunotherapy or vaccination. These pseudopeptides have a size of at least 6 amino acids comprising at least one unit chosen from the B units of general formula I and/or II defined below:



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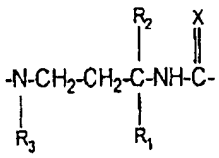


(II)

in which:

R_1, R_2 and R_3 each independently of one another represent an amino acids side chain and may be identical or different, and

X represents an oxygen or sulfur atom, preferably an oxygen atom.

Advantageously, R_2 represents a hydrogen atom.

The expression amino acids is understood to mean the primary amino acids which encode proteins, the amino acids derived after enzymatic action such as trans-4-hydroxyproline and the natural amino acids but which are not present in proteins, such as norvaline, N-methyl-L-

leucine, staline (Hunt S. in Chemistry and Biochemistry of the amino acids, Barett G.C., ed., Chapman and Hall, London, 1985), the amino acids protected by chemical functional groups which can be used in synthesis on solid supports or in liquid phase and the non-natural amino acids. Examples of these non-natural amino acids are given in the Novabiochem catalog (Catalog & Peptide synthesis Handbook; 1999; CH-4448, Läufelfinfgen, Switzerland) or the Néosystem catalog (Catalog 1997/1998; 67100 Strasbourg, France).

PSEUDOPEPTIDE, PROCEDE DE SYNTHESE, REACTIF ET APPLICATIONS

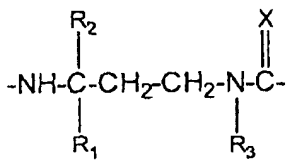
Depuis des années de nombreuses équipes se sont attachées à synthétiser des analogues de peptides ou de protéines qui miment les activités biologiques des peptides ou protéines naturels. On peut citer à titre d'exemple les analogues peptidiques obtenus par remplacement d'un ou plusieurs acides aminés de la série L par un ou des acides aminés correspondants de la série D, les peptides présentant une modification au niveau d'au moins une des liaisons peptidiques, telles que les liaisons rétro, inverso, rétro-inverso, carba et aza.

La liaison carba (CH_2-CH_2) a été décrite comme un mime potentiel de la liaison peptidique (Mendre C. et al., European J. Pharmacol., 186, p213-222, 1990; Attwood et al., Bioorg. Med. Chem. Lett., 7, p429-432, 1997). Par ailleurs, le remplacement du carbone α par un atome d'azote partiel ou complet sur un peptide a permis d'obtenir des pseudopeptides intéressants dénommés azapeptides et azatides respectivement (Gante, J., Synthesis, p405-413, 1989 ; Han H. et Janda K.D., J. Amer. Chem. Soc, 118, p2539-2544, 1996).

D'une manière générale ces analogues peptidiques, dénommés pseudopeptides, présentent comme premier avantage une stabilité métabolique supérieure à celle des peptides ou protéines naturels en raison du fait qu'ils ne sont pas dégradés par les protéases naturelles ou le sont moins vite. Par ailleurs, les changements de conformation induits par ces modifications chimiques peuvent améliorer les propriétés biologiques de ces pseudopeptides, voir par exemple les analogues décapeptidiques antagonistes des hormones hypothalamiques décrits dans WO-A-92/13883.

utilisés possèdent toujours une taille supérieure d'au moins 12 acides aminés (D. Osmanov, AIDS, 5(1), WHO1-WHO9, 1991). Dans un autre exemple comme le diagnostic de la maladie de Chagas, les peptides utilisés comportent au minimum 12 acides aminés (WO-A-97/18475). Dans (Bradshaw C.G. et col., J. Med. Chem.,37,1991-1995,1994) des sondes fluorescentes analogues de l'antagoniste heptapeptidique de NK_2, ont été obtenues par substitution d'un acide aminé et couplage avec un fluorophore.

C'est l'objet de la présente invention que de décrire une nouvelle famille de pseudopeptides comportant un nouveau motif carbaza modifiant de manière significative le squelette peptidique et dont la mise en oeuvre dans le cadre de la synthèse de peptides soit aisée aussi bien en phase solide qu'en phase liquide et ce, même pour des peptides de taille importante et notamment supérieure à 6 acides aminés. Cette nouvelle famille de pseudopeptides est utilisable dans le domaine diagnostique pour fournir des méthodes de diagnostic in vitro de pathologies associées à la présence de protéines endogènes ou exogènes chez un individu, ou dans le domaine thérapeutique et notamment l'immunothérapie ou la vaccination. Ces pseudopeptides ont une taille d'au moins 6 acides aminés comprenant au moins un motif choisi parmi les motifs B de formule générale I et/ou II définies ci-dessous :



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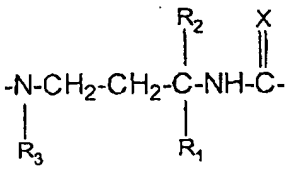

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dans lesquelles :

PSEUDOPEPTIDE, SYNTHESIS METHOD, REAGENT AND APPLICATIONS

For many years, many teams have focused on synthesizing analogs of peptides or proteins which mimic the biological activities of natural peptides or proteins. There may be mentioned, by way of example, the peptide analogs obtained by replacing one or more amino acids of the L series with one or more corresponding amino acids of the D series, the peptides exhibiting a modification at the level of at least one of the peptide bonds, such as the retro, inverso, retro-inverso, carba and aza bonds.

The carba bond (CH_2-CH_2) has been described as a potential mimic of the peptide bond (Mendre C. et al., European J. Pharmacol., 186, p. 213-222, 1990; Attwood et al., Bioorg. Med. Chem. Lett., 7, p. 429-432, 1997). Moreover, the partial or complete replacement of the α-carbon by a nitrogen atom on a peptide has made it possible to obtain advantageous pseudopeptides called azapeptides and azatides respectively (Gante, J., Synthesis, p. 405-413, 1989; Han H. and Janda K.D., J. Amer. Chem. Soc, 118, p. 2539-2544, 1996).

In general, these peptide analogs, called pseudopeptides, have, as a first advantage, a metabolic stability which is greater than that of natural peptides or proteins because they are not degraded by natural proteases or are degraded less rapidly. Moreover, the conformational changes induced by these chemical modifications can improve the biological properties of these pseudopeptides.

While the techniques for the synthesis of so-called natural peptides, in particular on solid supports, are well established and make it possible to easily prepare peptides comprising several tens of amino acids, the

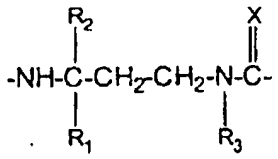
introduction of these modifications in order to prepare pseudopeptides renders the synthesis more complex, in particular for long pseudopeptides.

Moreover, in the field of immunology and both in the diagnosis of viral or autoimmune diseases and in immunotherapy or vaccination, the synthetic peptides mimicking the epitopes of proteins represent a valuable alternative. The size of the peptides which are analogs of these antigenic determinants or epitopes is an important factor in the choice of these peptides and has been the subject of numerous publications (M.H.V. Regenmortel, Immunology Today, 10(8), p. 266-271, 1989 or M.H.V. Regenmortel, Biomedical Peptides, Proteins & Nucleic Acids, 1, p. 109-116, 1995). While originally it was accepted that an epitope comprises between 15 and 22 amino acids, recent studies show that this size may be reduced to a few amino acids. In the immunity domain, crystallographic studies on the interaction of peptides and the major histocompatibility complex (MHC) indicate a size of 9 to 13 amino acids for a good interaction with the MHC class I molecules and 9 to 25 for the MHC class II (H.G. Rammensee, Current Opinion in Biotechnology, 7, p. 85-96, 1995). Likewise, in diagnosis, the size is a critical factor for the use of peptides. In the case of HIV (human immunodeficiency virus), the smallest epitopes comprise from 4 to 6 amino acids but the peptides used still have a size greater than at least 12 amino acids (D. Osmanov, AIDS, 5(1), WHO1-WHO9, 1991). In another example, such as the diagnosis of Chagas' disease, the peptides used comprise a minimum of 12 amino acids (WO-A-97/18475).

It is the object of the present invention to describe a novel family of pseudopeptides comprising a novel carbaza unit significantly modifying the peptide backbone and whose use in the context of peptide synthesis is easy both in solid phase and in liquid phase, and this even for peptides of a large size and

in particular greater than 6 amino acids. This novel family of pseudopeptides can be used in the diagnostic field to provide in vitro methods for the diagnosis of pathology conditions associated with the presence of endogenous or exogenous proteins in an individual, or in the therapeutic field, and in particular immunotherapy or vaccination.

These pseudopeptides have a size of at least 6 amino acids comprising at least one unit chosen from the B units of general formula I and/or II defined below:



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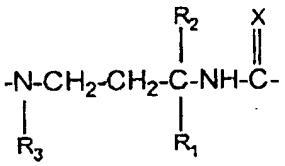

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in which:

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